

We claim:

1. A method of simultaneously determining a signature sequence for each polynucleotide in a population of polynucleotides, the method comprising the steps of:

5 attaching an oligonucleotide tag from a repertoire of tags to each polynucleotide of the population to form tag-polynucleotide conjugates such that substantially every different polynucleotide has a different oligonucleotide tag attached;

generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment of the same size ladder having an end and the same oligonucleotide tag as every other polynucleotide fragment of the size ladder;

separating the polynucleotide fragments into size classes;

labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides at the end of such polynucleotide fragment;

15 copying the labeled oligonucleotide tags of each polynucleotide fragment of each size class; and

separately hybridizing the labeled oligonucleotide tags of each size class with their respective complements under stringent hybridizaion conditions, the respective complements being attached as populations of substantially identical oligonucleotides in spatially discrete and addressable regions on one or more solid phase supports, and the respective signature sequences being determined by the sequence of labels associated with each spatially discrete and addressable region of the one or more solid phase supports.

2. The method of claim 1 wherein said steps of generating and separating further include forming a plurality of aliquots of tag-polynucleotide conjugates, and shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount.

3. The method of claim 2 wherein said step of shortening is carried out enzymatically with a type IIs restriction endonuclease.

4. The method of claim 1 wherein said step of generating further includes forming extension products of known lengths for each tag-polynucleotide.

5. The method of claim 4 wherein said step of generating further includes extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing

oligonucleotide and then extending the initializing oligonucleotide by ligating extension oligonucleotides.

6. A method of simultaneously determining a signature sequence of each polynucleotide in a sample of tag-polynucleotide conjugates wherein substantially every different polynucleotide has a different tag, the method comprising the steps of:

generating a size ladder for every tag-polynucleotide conjugate such that each size ladder has a plurality of size classes of polynucleotide fragments;

separating the size classes of polynucleotide fragments;

amplifying and labeling the tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of each such polynucleotide fragment;

separately hybridizing the labeled tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached to each of a plurality of microarrays, each microarray of the plurality having the same spatially addressable hybridization sites; and

determining each signature sequence in the sample by a set of signals generated at hybridization sites having the same address on each of the plurality of microarrays.

7. The method of claim 6 wherein said step of generating further includes generating said size ladder for said every tag-polynucleotide conjugate to form a mixture of said size classes.

8. The method of claim 7 wherein said step of separating further includes forming substantially homogeneous populations of each of said size classes of said mixture by physical separation.

9. The method of claim 8 wherein said step of generating further includes extending a first primer to copy said tag of each tag-polynucleotide conjugate to form an initializing oligonucleotide and then extending the initializing oligonucleotide by ligating extension oligonucleotides.

10. The method of claim 9 wherein said step of forming by said physical separation is carried out by preparative gel electrophoresis or HPLC.

11. The method of claim 10 wherein said extension oligonucleotides have a length of from 2 to 10 nucleotides.

12. The method of claim 11 wherein said extension oligonucleotides have a length of from 4 to 6 nucleotides.

13. The method of claim 12 wherein said step of forming by said physical separation is
5 carried out by denaturing HPLC.

14. The method of claim 13 wherein said extension oligonucleotides comprising one or more degeneracy-reducing nucleotide analogs.

10 15. A kit for simultaneously determining a signature sequence of each polynucleotide in a sample of tag-polynucleotide conjugates wherein substantially every different polynucleotide has a different tag, the kit comprising:

a vector containing a repertoire of oligonucleotide tags for forming tag-polynucleotide conjugates;

15 extension oligonucleotides for extending an initializing oligonucleotide to generate size ladders of polynucleotide fragments; and
a plurality of microarrays of tag complements.

20 16. The kit of claim 15 further including labeling means for copying and labeling said oligonucleotide tags.